und damit der Grundrate des axoplasmatischen Flusses entspricht[1,9]. Nach 2d hat das Tectum zwar noch eine höhere Aktivität als das prätectale Segment, was jedoch mit der RNS-Eigensynthese der Zellkörperschicht im Tectum aus über das Blut herangeführtem 3-H-Uridin zu erklären ist (Figur 1). Aber nach 4d ist das 3. Segment schon so stark markiert wie das Tectum, und nach 8d herrscht im optischen System ein gleichmässig abfallender Gradient der Aktivität: der Fluss markierter Substanzen hat das 3. Segment erreicht. Nach 16d ist die Aktivität der distalen Bereiche des Tractus opticus erheblich angestiegen. Im nicht injizierten Tractus hingegen bleibt die Aktivität aller 4 Segmente gegenüber der des augennahen 1. Segments des injizierten Tractus gleichbleibend sehr gering, was eindeutig auf einen intraaxonalen Fluss im injizierten Nerven hinweist und eine extraaxonale Ausbreitungsweise ausschliesst.

Auch autoradiographisch lässt sich schon nach 12d in den optischen Faserschichten des mit dem injizierten Auge zusammenhängenden (kontralateralen) Tectum opticum eine höhere Aktivität nachweisen als in den übrigen Schichten (Figur 2). Die selektive Markierung der optischen Schichten im Tectum, die die terminalen Bereiche der optischen Axone enthalten, gilt nach intraocularer Tracer-Applikation als sicheres Kriterium des intraaxonalen Flusses dieses Tracers[8].

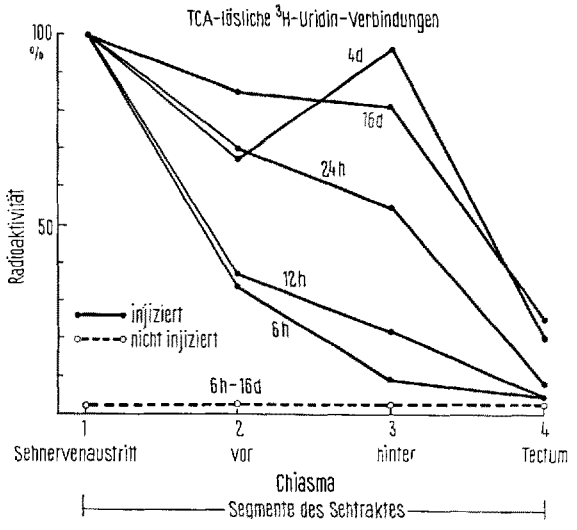

Fig. 3. Darstellung der Radioaktivitätsverteilung Trichloressigsäure-(TCA)-löslicher 3-H-Uridin-Verbindungen im Tractus opticus des inj. bzw. nicht inj. Systems von *Scardinius erythrophthalmus* nach verschiedenen Incorporationszeiten. Die Markierung bleibt, wie die der RNS (vgl. hierzu Figur 1), auf das inj. System beschränkt, was einen intraaxonalen Transport niedermolekularer Uridin-Verbindungen anzeigt.

Im Gegensatz zu früheren Befunden[10], bei denen für die Ermittlung eines Stofftransportes im optischen System der Teleosteer lediglich das Tectum opticum und nicht auch das prätectale Segment mit dem ersten Segment des Tractus opticus verglichen wurde, erbrachten die vorliegenden Untersuchungen, dass sich neben den 3-H-Uridin-markierten RNS-Verbindungen auch TCA-lösliche, d.h. niedermolekulare 3-H-Uridin-Verbindungen intraaxonal ausbreiten (Figur 3). Ähnlich wie im Falle der Ausbreitung der hochmolekularen RNS bleibt auch bei den löslichen 3-H-Uridin-Verbindungen die Markierung weitestgehend auf das mit dem injizierten Auge in Verbindung stehende optische System beschränkt. Die Aktivität im nicht injizierten Trakt entspricht der des Cerebellums, was auf eine Ausbreitung eines Teiles des Tracers über die Blutbahn zurückzuführen ist. Aus dem Befund, dass die Radioaktivität der TCA-löslichen Verbindungen im kontralateralen Tectum nach 12 h p.i. deutlich über der des Cerebellums sowie vor allem des ipsilateralen Tectums liegt, ist eine Geschwindigkeit der intraaxonalen Ausbreitung für die löslichen Uridin-Verbindungen von etwa 30–50 mm/d abzuleiten.

Der Anteil des in hochmolekulare RNS-Verbindungen eingebauten 3-H-Uridins an der Gesamtaktivität ändert sich mit zunehmenden Incorporationszeiten: nach Kurzzeiten bis zu etwa 2d beträgt er lediglich bis zu 5%, um anschliessend nach Langzeiten bis auf maximal etwa 30% (nach 12d) anzusteigen.

Die vorliegenden Ergebnisse sprechen für einen intraaxonalen Transport sowohl von RNS (evtl. mitochondriale RNS?) als auch von deren niedermolekularen Vorstufen (evtl. Oligo- und Polynukleotide?) mit unterschiedlichen Geschwindigkeiten von 1,5 bzw. 50 mm/d, wie er bisher nur für lösliche bzw. Strukturproteine nachgewiesen wurde.

Weiterführende Untersuchungen hinsichtlich einer Lokalisation und Spezifizierung beider Fraktionen sind in Angriff genommen[11].

Summary. After application of 3-H uridine to one eyeball, an intraaxonal flow of RNA (1–3 mm/d) and low-molecular uridine-compounds (30–50 mm/d) has been demonstrated in the optic tract of *Scardinius erythrophtalmus* and *Carassius carassius* (Teleostei).

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Uptake from Blood and Conjugation of Bilirubin by the Intestinal Mucosa of Wistar, Gunn and Hepatectomized Rats

A direct transfer of unconjugated bilirubin from blood to the bowel lumen across the intestinal wall has been reported in Gunn rats[1,2], in patients with the Crigler-Najjar syndrome[1] and in Wistar rats treated with α-naphthylisothiocyanate[3,4]. On the other hand, both conjugated and unconjugated bilirubin may be absorbed from the gut lumen[5]. Experiments performed in Gunn rats indicated that at least in this mutant strain, conjugated bilirubin was hydrolized previously to absorption, either by bacterial β-glucuronidase or by intrinsic β-glucuronidase localized in the intestinal mucosa[6].

In vitro studies seem to confirm that bidirectional transport of unconjugated bilirubin across the intestinal wall may be accomplished by passive diffusion[7]. It has

been also described by in vitro experiments that the intestinal mucosa is able to form glucuronide conjugates[8] including bilirubin as a substrate[9]. Furthermore glucuronide synthesis by the intestinal mucosa appears to be parallel to the liver capacity and when the latter is diminished, as observed in Gunn rats, deficiency of the former has been also reported[8].

In the present work it was demonstrated that, when unconjugated bilirubin was continuously infused i.v. to rats, conjugated bilirubin was always detected in the intestinal mucosa. In Wistar rats subjected to total hepatectomy after derivation of the portal blood to the femoral vein, we also observed conjugated bilirubin after infusion of the unconjugated pigment. On the contrary, in the intestinal mucosa of Gunn rats thus perfused, no conjugated bilirubin could be found.

Methods. The bile duct of Wistar, heterozygous and homozygous Gunn rats of both sexes was cannulated with polyethylene tubing (Intramedic PE 50) under ether anesthesia. A second catheter (Intramedic PE 10) was placed into the femoral vein and then the animals were maintained in restraining cages. A solution of unconjugated bilirubin (Sigma Chemical Co), 2 mg/100 g body weight was administered i.v. followed by a continuous infusion of bilirubin at a rate of 100 µg/min per 100 g body weight for 70 min. Bile was collected at 10 min intervals and then diluted with water for determination of bilirubin concentration[10].

In another group of Wistar rats, a third heparinized polyethylene catheter (1 mm in internal diameter and 13 cm in length) was fixed, one end into the right femoral vein and the other one, previous laparotomy, into the portal vein. The latter was distally directed and fixed with 2 ligatures whereas the whole hepatic pedicle including the bile duct was ligated close to the liver.

Total hepatectomy was performed after derivation of the portal blood. Unconjugated bilirubin solution (2 mg/100 g body weight) was injected i.v., followed by a continuous infusion of 5% glucose solution. In 3 of these experiments, crystalline ^{14}C-bilirubin[11] with a specific activity of 2,400 to 4,200 dpm/µg was injected 5 min after the injection of the unlabelled pigment in a total dose of 200,000 to 400,000 dpm. Hepatectomized rats were killed at different time intervals (Table II). In all the rats blood

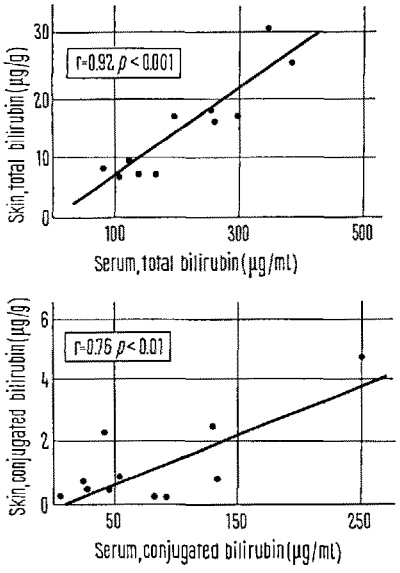

Fig. 1. Relationship between serum bilirubin and bilirubin determined in skin homogenates of intact rats infused with unconjugated bilirubin. 4 g of skin with the hairs clipped off, were homogenized in 20 ml pH 2.2 citric acid-phosphate buffer.

Table I. Bilirubin determined in the intestinal mucosa as compared with T_m values in rats infused with unconjugated bilirubin

Rats	Liver capacity for bilirubin excretion T_m (µg/100 g body wt./min)	Bilirubin determined in the intestinal mucosa	
		Azopiment *B* (%)	Concentration of bilirubin (µg/g)
Wistar	77	46	6
Wistar	86	64	23
Wistar	84	78	12
Heterozygous Gunn	39	10	15
Heterozygous Gunn	44	18	17
Heterozygous Gunn	39	35	17
Homozygous Gunn	0.8	0	12
Homozygous Gunn	1.9	0	20

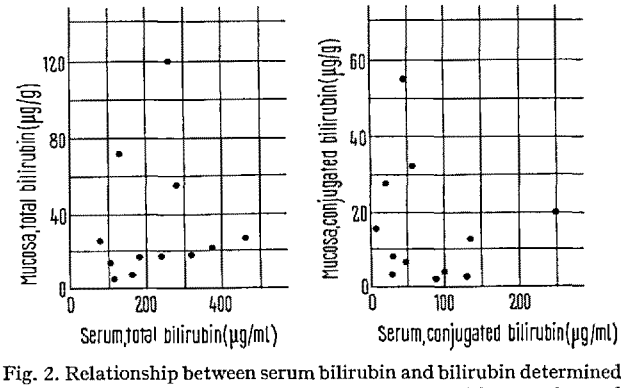

Fig. 2. Relationship between serum bilirubin and bilirubin determined in mucosal homogenates of intact rats infused with unconjugated bilirubin. 500 mg of mucosa were homogenized in 2.5 ml pH 2.2 citric acid-phosphate buffer.

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was collected and dorsal skin as well as the small intestine were removed at the end of the experiment. The intestinal contents was collected by washing out and the mucosa was stripped by scraping, blotted dry and weighed.

Total bilirubin was determined in bile and serum samples by diazoreaction[12]. It was measured in skin and mucosa homogenates and in the intestinal contents, by a modification of HARGREAVES' method[13], with the use of diluted diazoreactive[12]. Radioactivity was measured according to previous methods[14]. Serum samples, intestinal contents and both skin and mucosa homogenates were diazotized, concentrated to a small volume[15] and chromatographed on paper[16]. Densitometry of azopigments was then carried out (Densicord, 542A, Photovolt). In the experiments where radioactive bilirubin was administered, scanner of the paper was also performed (4π Scanner, Tracerlab). Conjugated bilirubin was calculated from total bilirubin values and the proportion of azopigment B on the chromatograms.

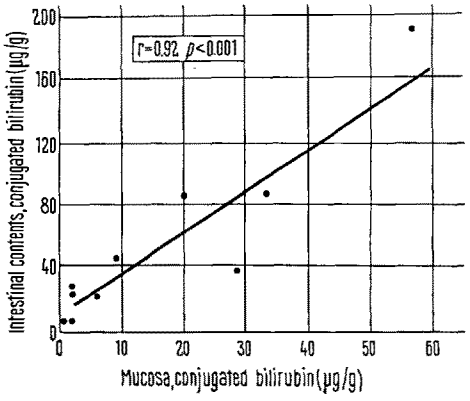

Fig. 3. Relationship between conjugated bilirubin determined in mucosal and intestinal contents homogenates, of rats infused with unconjugated bilirubin. 1 g of intestinal contents was homogenized in 5 ml pH 2.2 citric acid-phosphate buffer.

Table II. Determination of bilirubin concentration and proportion of azopigment B in the intestinal mucosa of untreated and hepatectomized Wistar rats. The latter received (2 mg/100 g body wt) unconjugated bilirubin

Rats	Bilirubin determined in the intestinal mucosa		Duration of the experiment after bilirubin injection (min)
	Concentration of bilirubin (µg/g)	Azopigment *B* (%)	
Untreated	0	0	–
Untreated	0	35	–
Untracted	0	0	–
Untreated	0	0	–
Untreated	0	0	–
Untreated	0	0	–
Untreated	1.6	0	–
Untreated	0	0	–
Untreated	0	0	–
Hepatectomized	not measured	71	5
Hepatectomized	10.8	31	7
Hepatectomized	21.5	75	10
Hepatectomized	8.2	43	10
Hepatectomized	8.3	61	18
Hepatectomized	25.0	9	25
Hepatectomized	20.8	62	25
Hepatectomized	7.2	11	25
Hepatectomized	not measured	43	30
Hepatectomized	15.0	54	40

Results. The results showed that when unconjugated bilirubin was infused in untreated rats, a positive significative correlation was found between conjugated and unconjugated serum bilirubin and both forms of pigment in skin homogenates (Figure 1). No correlation was found between serum pigments and those present in the intestinal mucosa (Figure 2). In this latter, variable amounts of azopigment B were seen in correspondence with T_m values (Table I). A positive correlation was observed between conjugated bilirubin of intestinal mucosa and lumen contents (Figure 3). In hepatectomized rats azopigment B was not detected in serum samples but was always present in the intestinal mucosa (Table II). Mesenteric fat of hepatectomized rats contained only azopigment A, whereas that of 2 rats with bile duct ligation contained azopigment A and B.

When radioactive unconjugated bilirubin was injected in hepatectomized rats, scanner of chromatograms of intestinal mucosa revealed a peak of radioactivity coincident with azopigment B and a secondary peak with azopigment A. Azopigment B spots were pooled, eluted from paper and rechromatographed. The peak of radioactivity present in azopigment B was reproduced. When elution was again performed and submitted to acid hydrolysis[16] for 45 min and then chromatographed, 70% of azopigment A was now detected with most of the radioactivity present in this area.

It is concluded that serum unconjugated bilirubin can be taken up and then conjugated by the rat intestinal mucosa in vivo. It still remains to be demonstrated whether or not conjugated bilirubin may be transfered as such to the gut lumen[17].

Resumen. Se infundió bilirrubina no conjugada en tres grupos de ratas: normales, totalmente hepatectomizadas y Gunn homocigotas, observándose la aparición de bilirrubina conjugada en la mucosa intestinal de los dos primeros grupos. Dichos experimentos, confirmados con el uso de bilirrubina-^{14}C, demuestran que la bilirrubina no conjugada sérica puede ser captada y conjugada por la mucosa intestinal de la rata.

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